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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design and synthesis of 3-[3-(substituted phenyl)-4-piperidin-1-ylmethyl/-4-morpholin-4-ylmethyl-4,5-dihydro-isoxazol-5-yl]-1*H*-indoles as potent anti-inflammatory agents

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Abstract The synthesis of new indole derivatives bearing isoxazoline moiety (**3a–d** and **4a–d**) has been described. IR, ^{1}H NMR, and mass spectral data supported the structures of synthesized compounds. The compounds were tested in vivo for their anti-inflammatory activity by carrageenin-induced rat paw edema method. The compounds that showed good anti-inflammatory activity were screened for their ulcerogenic and lipid peroxidation activities. The most active compound of this series is 3-[3-(4-methoxyphenyl)- 4-morpholin-4-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1*H*-indole **4d**.

Keywords Indole · Isoxazoline · Anti-inflammatory · Ulcerogenicity · Lipid peroxidation

Introduction

Almost all nonsteroidal anti-inflammatory drugs (NSAIDs) under current clinical usage have aryl acetic acid pharmacophore and are highly acidic in nature. Therefore, long-term use of these drugs is associated with gastrointestinal ulceration, bleeding, and nephrotoxicity (Langman *et al*., 1994), thus indicating a clear need to develop nonacidic nonsteroidal anti-inflammatory agents. Two NSAIDs, indomethacin and tenidap, which are derivatives of heterocyclic indole nucleus (Fig. 1), are used clinically for the treatment of different anti-inflammatory disorders and are known to induce erosions and ulcers in the gastrointestinal tract (Hardman and Limbird, 2001; Moore *et al*., 1996; Yoshikawa *et al*., 1993). Indole derivatives have been reported to possess a wide variety of biological properties

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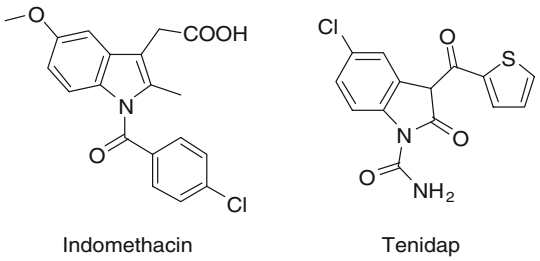


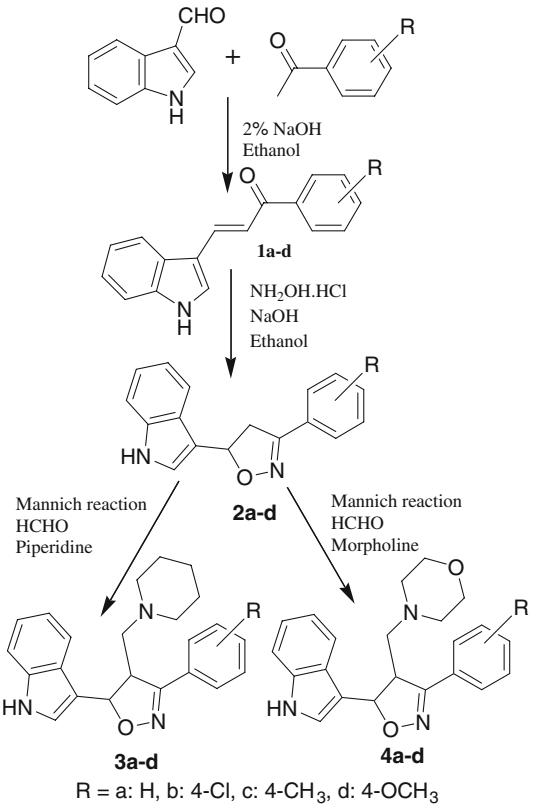
Fig. 1 Indomethacin and tenidap are known to induce erosions and ulcers in the gastrointestinal tract

viz., anti-inflammatory (Amir and Kumar, 2005; Amir *et al*., 2008; Singh *et al*., 2008), analgesic (Radwan *et al*., 2007), anticonvulsant (El-Gendy *et al*., 2008), and antibacterial (Gurkok *et al*., 2009; Yamamoto and Kurazono, 2007). Furthermore, substitution of heterocyclic moiety at 3-position of indole ring has been shown to improve anti-inflammatory activity (Radwan *et al*., 2007; Rani *et al*., 2004). Moreover, isoxazoline derivatives have been reported to possess potent protein tyrosine phosphatase inhibitor, and anti-inflammatory activity (Basappa *et al*., 2004; Habeeb *et al*., 2001; Maurya *et al*., 2008). Recently, *m*-terphenyl amines, which do not have traditional arylacetic acid moiety, have been reported to possess potent cyclooxygenase-inhibiting activity (Bauer *et al*., 2007). Encouraged by these observations and also in continuation of our search for potent anti-inflammatory molecules (Amir *et al*,. 2007a, b), we report the synthesis of new 3-substituted indole derivatives possessing isoxazoline moiety to get compounds of higher biological significance.

Results and discussion

Chemistry

In the present work, a series of eight new compounds were synthesized. Scheme 1 illustrates the synthetic route for the preparation of target compounds. 1-(Substituted phenyl)-3-(1*H*-indol-3-yl)-2-propen-1-ones **1a–d** were prepared by the method reported in literature (Amir *et al*., 2008). 3-[3-(Substituted phenyl)-4,5-dihydro-isoxazol-5-yl]-1*H*-indoles **2a–d** were obtained by cyclization of **1a–d** by treating with hydroxylamine hydrochloride in the presence of NaOH in absolute ethanol. The IR spectrum of compound **2b** showed absorption peaks at 1356 and 1652 cm^{-1} due to C–O–N and C=N stretching vibrations. The NH stretching vibration was obtained at 3310 cm^{-1}. The structure was further supported by its ^1H NMR spectrum, which showed two double doublets at δ 3.59 and δ 3.74 for CH_2 protons of isoxazoline ring. The CH proton at C-5 of isoxazoline was obtained as triplet at δ 6.08. Thus, disappearance of signals of the olefinic protons and appearance of CH_2 and CH proton signals in the spectrum confirmed the formation of isoxazoline ring. The NH proton of indole nucleus was obtained as a singlet at δ 8.17. The mass spectrum of the compound **2b** showed molecular ion peak M^+ at m/z 297 and M^++2 at m/z 299 corresponding to molecular formula $C_{17}H_{13}ClN_2O$. The



Scheme 1 Protocol for the synthesis of 3-[3-(substituted phenyl)-4- piperidin-1-ylmethyl/-4-morpholin-4-ylmethyl-4,5-dihydro-isoxazol-5-yl]-1*H*-indoles

indolylisoxazolines **2a**–**d** on treatment with piperidine in the presence of formaldehyde in ethanol gave corresponding 3-[3-(substituted phenyl)-4-piperidin-1-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1*H*-indoles **3a**–**d**. The IR spectrum of compound **3b** showed characteristic absorption peak at 3272 (NH), 1686 (C=N), 1362 cm^{-1} (C–O–N). The structure of the compound was further confirmed by its ^1H NMR spectrum, which showed a doublet at δ 5.58 for $–CH_2–N$ proton. The six protons (3 CH_2) of piperidine ring were obtained as a multiplet at δ 1.37–1.55, whereas the $N–(CH_2)_2$ protons of piperidine ring were observed as a multiplet at δ 2.51–2.56. The mass spectrum of compound **3b** showed molecular ion peak M^+ at m/z 394 and M^++2 at m/z 396 corresponding to molecular formula $C_{23}H_{24}ClN_3O$. 3-[3-(substituted phenyl)-4-morpholin-4-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1*H*-indoles **4a**–**d** were prepared by treating **2a**–**d** with morpholine in the presence of formaldehyde in ethanol. The IR spectrum of compound **4b** showed characteristic absorption peak at 3323 (NH), 1689 (C=N), 1359 (C–O–N). The structure of the compound was further confirmed by its ^1H NMR spectra, which showed a doublet at δ 5.53 for $–CH_2–N$ proton. The four protons ($CH_2–N–CH_2$) of morpholine ring were obtained as a triplet at δ 2.52, whereas the ($CH_2–O–CH_2$) protons of morpholine ring were observed as a triplet at δ 3.73. The mass spectrum of compound **4b**

showed molecular ion peak M^+ at m/z 396 and M^++2 at m/z 398 corresponding to molecular formula $C_{22}H_{22}ClN_3O_2$.

Pharmacology

All the newly synthesized compounds (**3a–d** and **4a–d**) were evaluated for their anti-inflammatory activity against carrageenin-induced paw edema method in rats, and compared with the reference drug indomethacin. Percent of the edema inhibition was calculated both after 3 and 4 h of carrageenin treatment. Both readings are reported in Table 1, but because % inhibition was found to be more significant after 4 h, the discussion is based on this reading. The tested compounds showed anti-inflammatory activity ranging from 45.45–55.55% (Table 1), whereas standard drug indomethacin showed 76.5% inhibition after 4 h. The anti-inflammatory activity of compounds **3a–d** having a piperidin-1-ylmethyl group at fourth position of isoxazoline ring was found in the range of 48.48–53.53%. Compound **3b** having 4-chlorophenyl group at third position of isoxazoline ring showed maximum activity (53.53%). Replacement of 4-chlorophenyl group by phenyl (**3a**), 4-methylphenyl (**3c**), and 4-methoxyphenyl (**3d**) groups resulted in a slight decrease in anti-inflammatory activity (48.48%, 51.51%, and 50.5% respectively). The anti-inflammatory activity of compounds **4a–d** having a morpholin-4-ylmethyl group at fourth position of isoxazoline ring was found between 45.45% and 55.55%. The highest activity (55.55%) was found in compound **4d** having 4-methoxyphenyl group at third position of isoxazoline ring. Replacement of this group by phenyl

Table 1 Anti-inflammatory, ulcerogenic, and lipid peroxidation activity of compounds **3a–d** and **4a–d**

Compound	Dose (mg kg^{-1})	Anti-inflammatory activity (% inhibition ± SEM)[a]		Dose (mg $kg^{-1)}$	Ulcerogenic activity (severity index ± SEM)[a]	nmol MDA content ± SEM/ 100 mg tissue[a]
		After 3 h (s)	After 4 h (s)			
3a	70	34.31 ± 2.36	48.48 ± 2.56*	–	–	–
3b	70	45.09 ± 1.96	53.53 ± 2.02*	210	0.583 ± 0.2*	4.38 ± 0.1*
3c	70	41.16 ± 2.14	51.51 ± 1.56*	210	0.5 ± 0*	4.28 ± 0.09*
3d	70	45.08 ± 1.96	50.5 ± 1.86*	210	0.75 ± 0.25**	4.51 ± 0.07*
4a	70	35.29 ± 2.14	45.45 ± 2.21*	–	–	–
4b	70	44.1 ± 2.51	50.5 ± 1.86*	210	0.75 ± 0.25**	4.39 ± 0.08*
4c	70	38.22 ± 2.51	48.48 ± 2.59*	–	–	–
4d	70	44.11 ± 2	55.55 ± 2.55*	210	0.25 ± 0.11*	4.16 ± 0.14*
IND	35	65.15 ± 1.51	76.5 ± 1.82	105	2.33 ± 0.17	9.29 ± 0.33
Control[b]	1 ml 0.5% CMC	–	–	1 ml 0.5% CMC	0	3.23 ± 0.05

IND indomethacin, *CMC* carboxymethyl cellulose

* $P < 0.0001$; ** $P < 0.001$

[a] Relative to standard and data were analyzed by Student's t test for $n = 6$

[b] The group was given orally 1 ml of 0.5% aqueous carboxymethyl cellulose solution

(**4a**), 4-chlorophenyl (**4b**), and 4-methylphenyl (**4c**) groups resulted in decrease of anti-inflammatory activity (45.45%, 50.5%, and 48.48% respectively).

The compounds **3b–d**, **4b**, and **4d**, which showed more than 50% anti-inflammatory activity, were further tested for their acute ulcerogenic activity. The tested compounds showed a significant reduction in ulcerogenic activity with the severity index ranging from 0.25 ± 0.11 to 0.75 ± 0.25, whereas the standard drug indomethacin showed a high severity index of 2.33 ± 0.17. The maximum reduction in ulcerogenic activity (0.25 ± 0.11) was found in compound **4d** having 4-methoxyphenyl group at position 3, and a morpholin-4-ylmethyl group at position 4 of isoxazoline ring. Rest of the tested compounds also showed better gastrointestinal safety profile compared with indomethacin (Table 1). It has been reported that compounds showing less ulcerogenic activity also show reduced malondialdehyde (MDA) content—a byproduct of lipid peroxidation (Pohle *et al*., 2001). Therefore, an attempt was made to correlate the decrease in ulcerogenic activity of the compounds with that of lipid peroxidation. All the compounds screened for ulcerogenic activity were analyzed for their effect on lipid peroxidation. Lipid peroxidation is measured as nanomoles of malondialdehyde (MDA) per 100 mg of gastric mucosa tissue. Indomethacin exhibited maximum tissue lipid peroxidation 9.29 ± 0.33, whereas the control group showed 3.23 ± 0.05. It was found that all the isoxazoline derivatives showing less ulcerogenic activity also showed reduction in lipid peroxidation (Table 1). Thus, these studies showed that the synthesized compounds have inhibited the induction of gastric mucosal lesions and the results further suggested that their protective effect might be related to the inhibition of lipid peroxidation in the gastric mucosa.

Conclusions

Various indolylisoxazoline derivatives were prepared with the objective of developing better anti-inflammatory molecules with minimum ulcerogenic activity. It was noted that five compounds—**3b–d**, **4b**, and **4d**—were found to have more than 50% anti-inflammatory activity compared with standard drug (indomethacin 76.5%), against carrageenin induced paw edema in rats. It was observed that indolylisoxazolines having a piperidin-1-ylmethyl group (**3a**, **3b**, **3c**) showed slightly improved anti-inflammatory activity than their respective isoxazoline derivatives having a morpholin-4-ylmethyl group (**4a**, **4b**, **4c**), except in compound **4d** for which the activity was found to be more (55.55%) than compound **3d** (50.5%). These compounds were tested for ulcerogenic activity and showed a significant reduction in severity index compared with standard reference drug. From these studies compound *3*-[3-(4-methoxyphenyl)-4-morpholin-4-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1*H*-indole **4d** has emerged as a lead compound, which showed maximum anti-inflammatory activity along with maximum reduction in ulcerogenic activity and lipid peroxidation. Thus, the series provided a new opportunity for possible modification of pharmacophoric requirements and future exploitation.

Experimental

Chemistry

Chemicals were purchased from E Merck (Germany), S. D. Fine Chemicals (India), and Qualigens (India). Melting points were determined in open capillary tubes and are uncorrected. Purity of the compounds was checked on TLC plates (silica gel G) and spots were located under iodine vapors or UV light. IR (KBr) spectra were recorded on a Nicolet, 5PC FTIR spectrometer (ν_{max} in cm^{-1}) and ^1H NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-300 (300 MHz FT NMR) spectrometer using TMS as internal reference (chemical shift in δ ppm). FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer. Elemental analyses were performed on a Carlo-Erba-1108 CHN elemental analyzer.

*General Procedure for the Preparation of 3-[3-(substituted phenyl)-4,5-dihydro-isoxazol-5-yl]-1H-indoles (**2a–d**).* To a solution of compounds **1a–d** (0.01 mol) in absolute ethanol (50 ml), hydroxylamine hydrochloride (0.01 mol) and solid NaOH (0.4 g) were added. The reaction mixture was refluxed for 4–6 h. The excess solvent was distilled off and crude product poured into ice water. The solids thus separated were filtered, washed with water, dried, and recrystallized from ethanol.

*3-[3- phenyl-4,5-dihydro-isoxazol-5-yl]-1H-indole (**2a**)*

Yield 56%; m.p. 166–167°C. IR (KBr, cm^{-1}): 3290 (NH), 1640 (C=N), 1350 (C–O–N). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.15 (1H, *s*, NH of indole), 6.97–7.63 (10H, *m*, ArH), 5.98 (1H, *t*, $J = 9.7$ Hz, CH), 3.71 (1H, *dd*, $J = 11.3$, 5.7 Hz, CH), 3.56 (1H, *dd*, $J = 9.2$, 7.6 Hz, CH); MS: m/z 262 (M^+). Elemental analysis: Calc. for $C_{17}H_{14}N_2O$: C, 77.84; H, 5.38; N, 10.68; found: C, 77.63; H, 5.16; N, 10.54%.

*3-[3-(4-Chlorophenyl)-4,5-dihydro-isoxazol-5-yl]-1H-indole (**2b**)*

Yield 72%; m.p. 186–188°C. IR (KBr, cm^{-1}): 3310 (NH), 1652 (C=N), 1356 (C–O–N). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.17 (1H, *s*, NH of indole), 7.09–7.68 (9H, *m*, ArH), 6.08 (1H, *t*, $J = 9.9$ Hz, CH), 3.74 (1H, *dd*, $J = 11.1$, 5.4 Hz, CH), 3.59 (1H, *dd*, $J = 9.0$, 7.5 Hz, CH); MS: m/z 297 (M^+), 299 (M^++2). Elemental analysis: Calc. for $C_{17}H_{13}ClN_2O$: C, 68.81; H, 4.42; N, 9.44; found: C, 68.59; H, 4.31; N, 9.31%.

*3-[3-(4- Methylphenyl)-4,5-dihydro-isoxazol-5-yl]-1H-indole (**2c**)*

Yield 69%; m.p. 207–208°C. IR (KBr, cm^{-1}): 3284 (NH), 1646 (C=N), 1343 (C–O–N). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.14 (1H, *s*, NH of indole,). 6.96–7.62 (9H, *m*, ArH), 5.95 (1H, *t*, $J = 9.7$ Hz, CH), 3.73 (1H, *dd*, $J = 11.4$, 5.8 Hz, CH), 3.55 (1H, *dd*, $J = 9.4$, 6.9 Hz, CH), 2.23 (3H, *s*, CH_3); MS: m/z 276 (M^+). Elemental analysis: Calc. for $C_{18}H_{16}N_2O$: C, 78.24; H, 5.84; N, 10.14; found: C, 78.06; H, 5.72; N, 10.03%.

3-[3-(4-Methoxyphenyl)-4,5-dihydro-isoxazol-5-yl]-1H-indole ***(2d)***

Yield 57%; m.p. 174–176°C. IR (KBr, cm^{-1}): 3297 (NH), 1650 (C=N), 1353 (C–O–N). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.12 (1H, *s*, NH of indole), 6.93–7.58 (9H, *m*, ArH), 5.92 (1H, *t*, $J = 9.9$ Hz CH), 3.82 (3H, *s*, OCH_3), 3.69 (1H, *dd*, $J = 11.6$, 6.1 Hz, CH), 3.51 (1H, *dd*, $J = 8.9$, 7.2 Hz, CH). MS: m/z 292 (M^+). Elemental analyses: Calc. for $C_{18}H_{16}N_2O_2$: C, 73.95; H, 5.52; N, 9.58; found: C, 73.78; H, 5.41; N, 9.43%.

General procedure for the preparation of 3-[3-(substituted phenyl)-4-piperidin-1-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1H-indoles ***(3a–d)***. Compounds **2a–d** (0.001 mol) were dissolved in a mixture of ethanol and dioxane (2:1). Then formaldehyde (40%, 1.5 ml) and piperidine (0.001 mol) in ethanol were introduced into the solution. The reaction mixture was stirred for 2 h and then refluxed for 4 h on a water bath. The resulting solution was concentrated, cooled, and poured into crushed ice. The solids thus separated were filtered, washed with water, dried, and recrystallized from ethanol.

3-[3- Phenyl-4-piperidin-1-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1H-indole ***(3a)***

Yield 69%; m.p. 105–107°C. IR (KBr, cm^{-1}): 3263 (NH), 2989 (C–H), 1680 (C=N), 1350 (C–O–N). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.23 (1H, *bs*, NH of indole), 6.91–7.47 (10H, *m*, ArH), 5.87 (1H, *d*, $J = 6.9$ Hz, CH), 5.51 (2H, *d*, $J = 6.3$ Hz, CH_2–N), 3.43–3.51 (1H, m, C*H*–CH_2–N), 2.43–2.47 (4H, *m*, CH_2–N–CH_2), 1.29–1.48 (6H, *m*, $3CH_2$); MS: m/z 359 (M^+). Elemental analysis: Calc. for $C_{23}H_{25}N_3O$: C, 76.85; H, 7.01; N, 11.69; found: C, 76.62; H, 6.87; N, 11.58%.

3-[3-(4- Chlorophenyl)-4-piperidin-1-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1H-indole ***(3b)***

Yield 60%; m.p. 76–77°C. IR (KBr, cm^{-1}): 3272 (NH), 2993 (C–H), 1686 (C=N), 1362 (C–O–N). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.41 (1H, *bs*, NH of indole). 7.08–7.67 (9H, *m*, ArH), 5.9 (1H, *d*, $J = 7.0$ Hz, CH), 5.58 (2H, *d*, $J = 6.2$ Hz, CH_2–N), 3.46–3.56 (1H, *m*, C*H*–CH_2-N), 2.51–2.56 (4H, *m*, CH_2–N–CH_2), 1.37–1.55 (6H, *m*, $3CH_2$); MS: m/z 394 (M^+), 396 (M^++2). Elemental analysis: Calc. for $C_{23}H_{24}ClN_3O$: C, 70.13; H, 6.14; N, 10.67; found: C, 69.84; H, 5.97; N, 10.83%.

3-[3-(4-Methylphenyl)-4-piperidin-1-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1H-indole ***(3c)***

Yield 63%; m.p. 124–126°C. IR (KBr, cm^{-1}): 3306 (NH), 3107 (C–H), 1684 (C=N), 1352 (C–O–N). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.32 (1H, *bs*, NH of indole). 6.71–7.48 (9H, *m*, ArH), 5.86 (1H, *d*, $J = 7.1$ Hz, CH), 5.36 (2H, *d*, $J = 6.2$ Hz, CH_2–N), 3.42–3.54 (1H, *m*, C*H*–CH_2–N), 2.47–2.53 (4H, *m*, CH_2–N–CH_2), 1.31–1.45 (6H, *m*, $3CH_2$), 1.25 (2H, *s*, CH_3), MS: m/z 373 (M^+). Elemental analysis: Calc. for $C_{24}H_{27}N_3O$: C, 77.18; H, 7.29; N, 11.25; found: C, 76.97; H, 7.16: N, 11.39%.

3-[3-(4-Methoxyphenyl)-4-piperidin-1-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1H-indole ***(3d)***

Yield 56%; m.p. 112–114°C. IR (KBr, cm^{-1}): 3298 (NH), 3011 (C–H), 1681 (C=N), 1356 (C–O–N). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.39 (1H, *bs*, NH of indole). 6.93–7.51 (9H, *m*, ArH), 5.89 (1H, *d*, $J = 6.9$ Hz, CH), 5.51 (2H, *d*, $J = 6.0$ Hz, CH_2–N), 3.81 (3H, *s*, OCH_3), 3.45–3.59 (1H, *m*, C*H*–CH_2–N), 2.49–2.57 (4H, *m*, CH_2–N–CH_2), 1.32–1.49 (6H, *m*, $3CH_2$); MS: m/z 389 (M^+). Elemental analysis: Calc. for $C_{24}H_{27}N_3O_2$: C, 74.01; H, 6.99; N, 10.79; found; C, 73.83; H, 6.87: N, 10.65%.

General procedure for the preparation of 3-[3-(substituted phenyl)-4-morpholin-4-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1H-indoles ***(4a–d)***. Compounds **2a–d** (0.001 mol) were dissolved in a mixture of ethanol and dioxane (2:1). Then formaldehyde (40%, 1.5 ml) and morpholine (0.001 mol) in ethanol were introduced into the solution. The reaction mixture was stirred for 2 h and then refluxed for 4 h on a water bath. The resulting solution was concentrated, cooled, and poured into crushed ice. The solids thus separated were filtered, washed with water, dried, and recrystallized from ethanol.

3-[3-(Phenyl)-4-morpholin-4-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1H-indole ***(4a)***

Yield 69%; m.p. 106–107°C. IR (KBr, cm^{-1}): 3295 (NH), 3016 (C–H), 1683 (C=N), 1344 (C–O–N): ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.98 (1H, *bs*, NH of indole), 7.13–7.74 (9H, *m*, ArH), 5.87 (1H, *d*, $J = 6.8$ Hz, CH), 5.49 (2H, *d*, $J = 6.2$ Hz, CH_2–N), 3.61 (4H, *t*, $J = 6.3$ Hz, CH_2–O–CH_2), 3.29–3.51 (1H, *m*, C*H*–CH_2–N), 2.46 (4H, *t*, $J = 4.6$ Hz, CH_2–N–CH_2); Mass: m/z 361 (M^+). Elemental analysis: Calc. for $C_{22}H_{23}N_3O_2$: C, 73.11; H, 6.41; N, 11.63; found: C, 72.89; H, 6.32: N, 11.46%.

3-[3-(4-Chlorophenyl)-4-morpholin-4-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1H-indole ***(4b)***

Yield 49%; m.p. 92–93°C. IR (KBr, cm^{-1}): 3323 (NH), 3026 (C–H), 1689 (C=N), 1359 (C–O–N): ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.41 (1H, *bs*, NH of indole), 7.16–7.78 (9H, *m*, ArH), 5.92 (1H, *d*, $J = 6.9$ Hz, CH), 5.53 (2H, *d*, $J = 6.2$ Hz, CH_2–N), 3.73 (4H, *t*, $J = 6.6$ Hz, CH_2–O–CH_2), 3.32–3.55 (1H, *m*, C*H*–CH_2–N), 2.52 (4H, *t*, $J = 4.5$ Hz, CH_2–N–CH_2); Mass: m/z 396 (M^+), 398 (M^++2). Elemental analysis: Calc. for $C_{22}H_{22}ClN_3O_2$: C, 66.75; H, 5.60; N, 10.61; found: C, 66.49; H, 5.48: N, 10.54%.

3-[3-(4-Methylphenyl)-4-morpholin-4-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1H-indole ***(4c)***

Yield 58%; m.p. 78–79°C. IR (KBr, cm^{-1}): 3246 (NH), 3012(C–H), 1690 (C=N), 1350 (C–O–N): ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.13 (1H, *bs*, NH of indole). 7.13–7.72 (9H, *m*, ArH), 6.02 (1H, *d*, $J = 7.0$ Hz, CH), 4.85 (2H, *d*, $J = 6.3$ Hz, CH_2–

N), 3.65 (4H, *t*, $J = 6.5$ Hz, CH_2–O–CH_2), 3.43–3.51 (1H, *m*, C*H*–CH_2–N), 2.25 (4H, *t*, $J = 4.4$ Hz, CH_2–N–CH_2), 1.26 (3H, *s*, CH_3), Mass: m/z 375 (M^+). Elemental analysis: Calc. for $C_{23}H_{25}N_3O_2$: C, 73.57; H, 6.71; N, 11.19; found: C, 73.41; H, 6.63: N, 11.07%.

3-[3-(4-Methoxyphenyl)-4-morpholin-4-ylmethyl)-4,5-dihydro-isoxazol-5yl]-1H-indole ***(4d)***

Yield 64%; m.p. 104–106°C. IR (KBr, cm^{-1}): 3291 (NH), 2987 (C–H), 1679 (C=N), 1346 (C–O–N): ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.26 (1H, *bs*, NH of indole). 7.14–7.79 (9H, *m*, ArH), 5.93 (1H, *d*, $J = 6.9$ Hz, CH), 5.46 (2H, *d*, $J = 6.0$ Hz, CH_2–N), 3.82 (3H, *s*, OCH_3), 3.67 (4H, *t*, $J = 6.9$ Hz, CH_2–O–CH_2), 3.49-3.57 (1H, *m*, C*H*–CH_2–N), 2.43 (4H, *t*, $J = 4.8$ Hz, CH_2–N–CH_2), Mass: m/z 391 (M^+). Elemental analysis: Calc. for $C_{23}H_{25}N_3O_3$: C, 70.57; H, 6.44; N, 10.73; found: C, 70.38; H, 6.31; N, 10.61%.

Pharmacology

Animals

Adult Wistar strain rats of either sex, weighing 150–200 g, were used for anti-inflammatory, ulcerogenic, and lipid peroxidation activities. The animals were allowed food and water ad libitum except during the experiments. They were housed in a room at 25 ± 2°C, and 50 ± 5% relative humidity with 12-h light/dark cycle. The animals were randomly allocated into groups at the beginning of all experiments. The experimental protocol was approved by the animal ethics committee of Hamdard University. All test compounds and the reference drug were administered orally, suspended in 0.5% carboxymethyl cellulose (CMC) solution.

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using the carrageenin induced paw edema method of Winter *et al.* (1962). The animals were randomly divided into groups of six and were fasted for 24 h before the experiment, with free access to water. Control group received 1 ml of 0.5% aqueous CMC. Each group was given the test compounds (70 mg/kg) and reference drug indomethacin (35 mg/kg) orally as a uniform suspension in 1 ml of 0.5% aqueous CMC according to the procedure of Radwan *et al.* (2007); 0.1 ml of carrageenin (1% solution in normal saline) was injected subcutaneously into the subplantar region of the right hind paw of each rat, 1 h after the administration of the test compounds and reference drug. The right hind paw volume was measured before and after 3 and 4 h of carrageenin treatment by means of a plethysmometer. The percent edema inhibition was calculated from the mean effect in the control and treated animals according to the following equation:

$$\text{Percent edema inhibition} = (V_c - V_t/V_c) \times 100$$

where, V_t represents the mean increase in paw volume in rats treated with test compounds and V_c represents the mean increase in paw volume in control group of rats.

Acute ulcerogenicity

Acute ulcerogenicity was determined according to method of Cioli *et al*. (1979). The animals were allocated into different groups of six each. Ulcerogenic activity was evaluated after oral administration of the test compounds at an oral dose of 210 mg/kg. The reference drug indomethacin was administered orally at a dose of 105 mg/kg. Control group received 1 ml of 0.5% CMC solution. Food, but not water, was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed with normal diet for 17 h and then killed. The stomach was removed and opened along the greater curvature, washed with distilled water, and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. For each stomach, the mucosal damage was assessed according to the following scoring system: 0.5: redness; 1.0: spot ulcers; 1.5: hemorrhagic streaks; 2.0: ulcers >3 but ≤5; 3.0: ulcers >5. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.

Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa *et al*. (1979). After screening for ulcerogenic activity, the gastric mucosa was scraped with two glass slides, weighed (100 mg), and homogenized in 1.8 ml of 1.15% ice cold KCl solution. The homogenate was supplemented with 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of acetate buffer (pH 3.5), and 1.5 ml of 0.8% thiobarbituric acid (TBA). The mixture was heated at 95°C for 60 min. After cooling the reactants were supplemented with 5 ml of the mixture of *n*-butanol and pyridine (15:1v/v), shaken vigorously for 1 min, and centrifuged for 10 min at 4000 rpm. The supernatant organic layer was taken out and absorbance was measured at 532 nm on UV spectrophotometer. The results were expressed as nanomol MDA per 100 mg tissue, using molar absorption coefficient $1.56 \times 10^5\ cm^{-1}\ M^{-1}$.

Statistical analysis

All values are expressed as mean ± SEM. Statistical significance was determined using Student's *t* test.

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